

Original Research Article

In vitro antidiabetic, anti-inflammatory and antioxidant potential of the ethanol extract of *Uromastix hardwickii* skin

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Abstract

Purpose: To evaluate the *in vitro* antidiabetic, anti-inflammatory and antioxidant potential of the ethanol extract of *Uromastix hardwickii* Skin (UHSEE).

Methods: The *in vitro* effects of UHSEE at various concentrations (10 - 250 µg/mL) on the activities of α -amylase, α -glucosidase and glucose uptake by yeast cells were used to evaluate its antidiabetic potential. Nitric oxide (NO), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide inhibitory assay were employed to determine its antioxidant effects, while the anti-inflammatory effects were evaluated using human red blood cell (HRBC) membrane stabilization assay.

Results: UHSEE inhibited α -amylase and α -glucosidase enzymes but increased glucose uptake by yeast cells in a concentration-dependent manner ($p < 0.05$). It also inhibited NO, DPPH, hydrogen peroxide and HRBC hemolysis in a concentration-dependent manner ($p < 0.05$).

Conclusion: *Uromastix hardwickii* skin exhibits promising good antidiabetic, antioxidant and anti-inflammatory properties *in vitro*. However, its true potentials in this regard needs to be evaluated *in vivo*.

Keywords: *Uromastix hardwickii* skin, Antidiabetic, Antioxidant, Anti-inflammatory, 2,2-Diphenyl-1-picrylhydrazyl, Membrane stabilization

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INTRODUCTION

Diabetes mellitus is a chronic metabolic and hormonal disorder characterized by hyperglycemia due to reduced insulin release or insulin resistance [1]. Effective management of hyperglycemia, especially postprandial hyperglycemia in diabetic patients has been a major challenge in the clinical treatment of

diabetes mellitus. Postprandial hyperglycemia is regulated by α -glucosidase and α -amylase, which catalyze the digestion of carbohydrate in the gut [2]. The down-regulation of α -glucosidase and α -amylase reduces the rate of glucose digestion and absorption, which ultimately diminishes the magnitude of postprandial hyperglycemia in both hyperglycemic and normoglycemic patients [3].

Hyperglycemia is associated with non-enzymatic glycation of protein molecules that lead to elevated production and release of reactive oxygen species (ROS) [4]. The ROS are implicated in the pathogenesis of diabetes mellitus and its complications as well as other disease conditions. They elicit release of inflammatory mediators, predisposes to insulin resistance and cardiovascular diseases. Diabetes has been described as a chronic inflammatory condition [5,6]. Owing to the above fact, the roles of anti-inflammatory agents and antioxidants in treating diabetes should not be under-estimated. The anti-inflammatory properties of common hypoglycemic drugs used in the clinical treatment of diabetes have been reviewed [6]. Their effects are not just via the reduction of plasma glucose level but also through the modulation of potent inflammatory mediators [6,7].

The use of natural products of either plant or animal origin in the traditional management of diabetes and other disease conditions is popular among Asians and Africans [8,9]. This is due to the high cost and unavailability of orthodox medicines as well as the perceived low side-effects, cultural acceptance and availability of traditional medicines [10,11]. One of the commonly used animal products in the ethnomedical treatment of diabetes in Pakistan is the skin of *Uromastyx hardwickii* [12].

Uromastyx hardwickii, popularly known as Hardwicke's spiny-tailed lizard or the Indian spiny-tailed lizard is a species of lizard belonging to the family Agamidae. It is a desert lizard found in both rocky and sandy environs of North Africa and the Middle East, especially India and Pakistan [13]. It is called "Barti chpkali" in Pakistan and the skin is used in the ethnomedical treatment of diabetes mellitus [12].

There is dearth of scientific evidence of its antidiabetic potential, hence this study evaluates the *in vitro* antidiabetic, antioxidant and anti-inflammatory properties of the ethanol extract of *Uromastyx hardwickii* skin.

EXPERIMENTAL

Collection of *Uromastyx hardwickii*

Four of *Uromastyx hardwickii* lizards were collected from desert and semi-desert areas of Pakistan. They were identified and the specimens were kept in the museum at Department of Zoology, Abdul Wali Khan University, Mardan khyber Puktunkhwa Pakistan. The lizards were handled according to the Guide

for the Use and Care of Laboratory Animals of National Research Council [14].

The experimental protocols was approved by the Ethics and Animal Care Committee, of Department of Zoology, Abdul Wali Khan University, Mardan khyber Puktunkhwa Pakistan (No: AWKUM/ZOO/2018/1770).

Preparation of the extract of *Uromastyx hardwickii* skin

Uromastyx Hardwickii were anesthetized with diethyl ether and then sacrificed. The skins were excised as described by Brito-Casillas *et al* [15]. They were dried under shade and ground into powder. About 200 g of the powdered sample was extracted with ethanol using Soxhlet apparatus for 8 h. The ethanol extract of *Uromastyx hardwickii* skin (UHSEE) was concentrated *in vacuo* using rotary evaporator at 40 °C.

Evaluation of *in vitro* antidiabetic

α -Glucosidase inhibitory activity

The α -glucosidase inhibitory activities of UHSEE and acarbose (10 - 250 μ g/mL concentration) were determined as described by Mopuri *et al* [16] without modification. The acarbose was used as the reference standard and the results were expressed as percentage inhibition of α -glucosidase activities. Same volume (0.25 mL) of different concentrations (10 - 250 μ g/mL) of UHSEE or acarbose were incubated with 0.50 mL α -glucosidase solution (1.0 U/mL in 0.1 M phosphate buffer of pH 6.8) at 37 °C for 15 min. Later, 0.25 mL of 4-nitrophenyl β -D-glucopyranoside (pNPG) solution (5 mM in 0.1 M phosphate buffer of pH 6.8) was added and further incubated at 37 °C for 20 min. The absorbance of the mixture was measured spectrometrically at 405 nm and the inhibitory activity was expressed in percentage.

Determination of α -amylase inhibitory activity

The inhibitory activity of various concentrations (10 - 250 μ g/mL) of UHSEE on α -amylase was measured using a spectrophotometer [17]. A known volume (0.25 mL) of different concentrations of UHSEE and miglitol were incubated with 0.50 mL of porcine pancreatic amylase (2 U/mL in 0.10 M phosphate buffer of pH 6.8) solution at 37 °C for 20 min. Later, 0.25 mL of starch (10 mg/mL in 0.10 M phosphate buffer of pH 6.8) was added to the mixture, which was incubated at 37 °C for 60 min. 1 mL of dinitrosalicylate colour reagent was added and

boiled for 10 min. The absorbance was determined at 540 nm. The results were presented as percentage inhibition of α -amylase activities and miglitol was used as reference standard. The percentage inhibition of α -glucosidase and α -amylase activities were calculated using Eq 1.

$$\text{Inhibition (\%)} = \frac{XA - YA}{XA} \times \frac{100}{I} \dots\dots\dots (1)$$

where XA = absorbance of control, YA = absorbance of test substance

Effect of UHSEE on glucose uptake by yeast cells

The effects of UHSEE and metformin (10 – 250 $\mu\text{g/mL}$) on glucose uptake by yeast cells (*Saccharomyces cerevisiae*) (Malteserkors, Denmark) were evaluated as described by Bhutkar and Bhise [18]. The yeast cells were washed by repeated centrifugation (4200 rpm, 5 min) in distilled water until the supernatant fluid were clear. 10 % (v/v) suspension of yeast was prepared in distilled water. 1 mL of glucose solution (25 mmol/L) was added to varied concentrations (10 - 250 $\mu\text{g/mL}$) of UHSEE and the mixture was incubated at 37 °C for 10 min.

After 10 min, 0.1 mL of the yeast suspension was added to the mixture, which was incubated (after thorough mixing) at 37 °C for 60 min. Later, the tubes containing the mixture were spun at 3800 rpm for 5 min in a centrifuge and the glucose concentration in the supernatant was determined. Each concentration was determined in triplicate and metformin was used as reference standard. The result is expressed as increase in glucose uptake (G) s shown in Eq 2 [18].

$$G (\%) = \frac{X - Y}{X} \times \frac{100}{I} \dots\dots\dots (2)$$

where X = absorbance of control, Y = absorbance of test substance

Evaluation of antioxidant activity

Nitric oxide (NO) radical scavenging assay

The NO radical scavenging assay of UHSEE was performed by monitoring the intensity of chromophore formed by reacting Griess reagent and nitrite liberated from sodium nitroprusside. Varied concentrations (10 - 250 $\mu\text{g/mL}$) of UHSEE were tested in triplicate as described by Bryan and Grisham [19]. The results are expressed as inhibition (H). Gallic acid was used as standard.

DPPH radical scavenging assay

The abilities of UHSEE to electron donation was evaluated with 1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol as described by Ak and Gulcin [20]. This protocol estimated the DPPH radical scavenging potential of UHSEE at various concentrations (10 - 250 $\mu\text{g/mL}$). The bleaching of the purple colour of DPPH solution to yellow was monitored spectrophotometrically at 517 nm after 30 min incubation in the dark at room temperature. Ascorbic acid was used as reference and the result was expressed as percentage free radical scavenging of DPPH.

$$H (\%) = \frac{A_0 - A_i}{A_0} \times \frac{100}{I} \dots\dots\dots (3)$$

where A₀ = absorbance of control; A_i = absorbance of test substance

Hydrogen peroxide scavenging assay

The ability of UHSEE to decompose hydrogen peroxide (H₂O₂) into water and oxygen gas was determined as described by Oktay *et al* [21] without modification. About 2.4 mL varied concentrations (10–250 $\mu\text{g/mL}$) of UHSEE were incubated with 0.6 mL H₂O₂ (2 mmol/L, in phosphate buffer of pH 7.4) for 10 min. The concentration of H₂O₂ was estimated spectrophotometrically at 230 nm and molar absorptive coefficient of 81 mol/L⁻¹/cm. Ascorbic acid was used as standard and the results are expressed as inhibition.

$$H (\%) = \frac{X_0 - X_i}{X_0} \times \frac{100}{I} \dots\dots\dots (4)$$

where X₀ = absorbance of control, X_i = absorbance of test substance

Determination of anti-inflammatory activity

Human red blood cell (HRBC) membrane stabilization assay

The effects of UHSEE on the hemolysis of human red blood cell (HRBC) in hypotonic saline solution was evaluated as described by Anosike *et al* [22]. Blood sample (5 ml) was collected from a healthy male donor (that has not received anti-inflammatory drug in the past 10 days) into EDTA sample bottle. The HRBC was repeatedly washed with normal saline by centrifugation as described by Anosike *et al* [22] until the supernatant was clear. Thereafter, 0.5 mL of 10 % suspension of the HRBC was added to test tubes containing different concentrations (5 – 250 $\mu\text{g/mL}$) of UHSEE dissolved in hypotonic saline solution in triplicate. The mixtures were

incubated for 30 min at 37 °C and later centrifuged at 3000 rpm for 5 min. The absorbance of the supernatants was recorded at 560 nm with spectrophotometer. Hypotonic solution was used as control while diclofenac (250 µg/mL) was used as reference standard. Inhibition (H) was calculated as in Eq 5.

$$H (\%) = \frac{AA - BB}{AA} \times \frac{100}{1} \dots\dots\dots (5)$$

where AA = absorbance of control, BB = absorbance of test substance

Heat-induced hemolysis assay

The effects of UHSEE on heat-induced hemolysis of HRBC was evaluated as described by Anosike *et al* [22]. The blood collection and preparation were as stated in the previous section. Thereafter 0.5 ml of 10 % suspension of the HRBC was added to test tubes containing different concentrations (5 – 250 µg/mL) of UHSEE dissolved in saline in triplicate. The mixtures were incubated for 30 min at 54 °C and later centrifuged at 3000 rpm for 5 min. The absorbance (ABS) of the supernatants was determined at 560 nm with spectrophotometer. Hypotonic solution was used as control while diclofenac (250 µg/mL) was used as reference standard. Inhibition (H) was computed as in Eq 6.

$$H (\%) = \frac{K_0 - K_u}{K_0} \times \frac{100}{1} \dots\dots\dots (6)$$

where Ko = absorbance of control, Ku = absorbance of test.

Statistical analysis

Data were subjected to ANOVA followed by Tukey’s multiple comparison test to determine significant difference using SPSS 22.0 software. *P* < 0.05 was considered sttistically significant.

RESULTS

Effect of UHSEE on α-glucosidase activity

The UHSEE and acarbose produced concentration-dependent inhibition of α-glucosidase activities (Figure 1). The α-glucosidase inhibitory activity of UHSEE was significantly (*p* < 0.05) lower compared to that of acarbose.

Effect of UHSEE on α-amylase activity

UHSEE and miglitol inhibited α-amylase activity in a concentration-dependent manner (Figure 2). The inhibitory effects of UHSEE and miglitol

against α-amylase activities were comparable except at 200 and 250 µg/mL where the former produced lower effects.

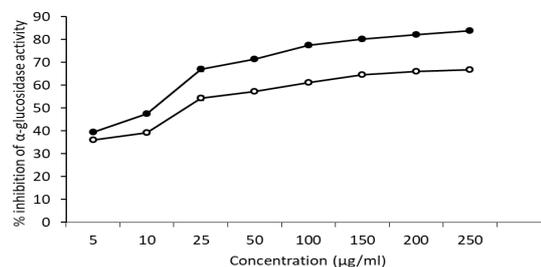


Figure 1: Effect of UHSEE on α-glucosidase activity. **Key:** -○-, UHSEE; -●-, Acarbose

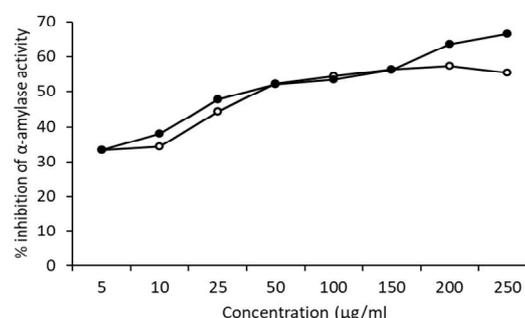


Figure 2: Inhibitory effect of UHSEE against α-amylase activities. **Key:** -○-, UHSEE; -●-, miglitol

Effect of UHSEE on glucose uptake by yeast cells

The UHSEE and standard inhibited glucose uptake by yeast cells in a concentration-dependent manner (Figure 3). The inhibitory effects of UHSEE and metformin were comparable.

Nitric oxide (NO) scavenging activity

Both UHSEE and gallic acid inhibited nitric oxide (NO) in a concentration-dependent manner. Their inhibitory properties against NO were comparable (Figure 4).

DPPH radical scavenging activity

The UHSEE and ascorbic acid produced concentration-dependent increase in the scavenging of DPPH radicals (Figure 5).

Hydrogen peroxide scavenging

UHSEE and ascorbic acid scavenged hydrogen peroxide in a concentration-dependent manner (Figure 6). The hydrogen peroxide scavenging ability of UHSEE was lower (*p* < 0.05) compared to ascorbic acid.

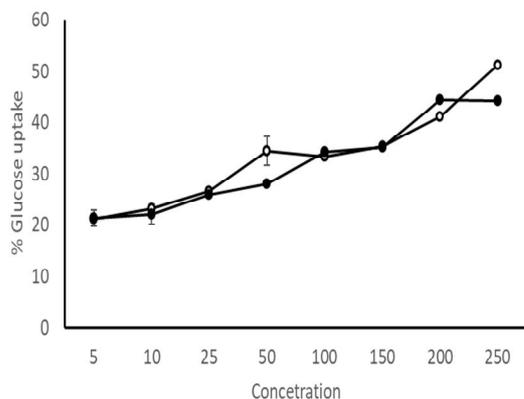


Figure 3: Effect of UHSEE on the uptake of glucose by yeast cells. Values are mean \pm SD of triplicate determinations. **Key:** -○-, UHSEE; -●-, Ascorbic acid

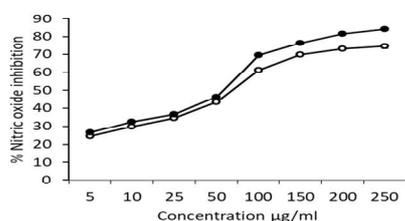


Figure 4: Inhibitory effect of UHSEE on nitric oxide (NO). **Key:** -○-, UHSEE; -●-, gallic acid

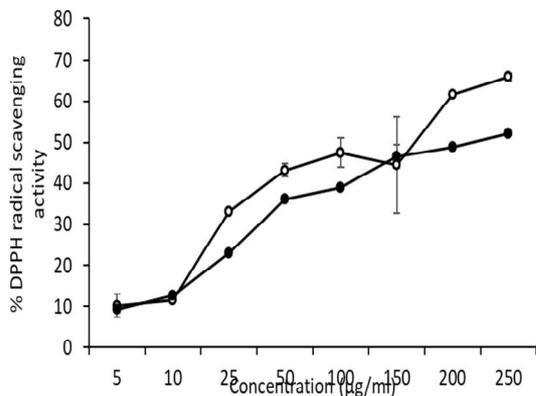


Figure 5: DPPH radical scavenging effect of UHSEE. **Key:** -○-, UHSEE; -●-, Ascorbic acid

Effect of UHSEE on HRBC-membrane stability

The USHEE inhibited HRBC hemolysis in a concentration-dependent manner. The anti-haemolytic activities of UHSEE at 150 – 250 µg/mL were comparable to diclofenac at 250 µg/mL (Table 1).

Effect of UHSEE on heat-induced haemolysis

UHSEE inhibited heat-induced hemolysis of HRBC in a concentration-dependent manner (Table 2).

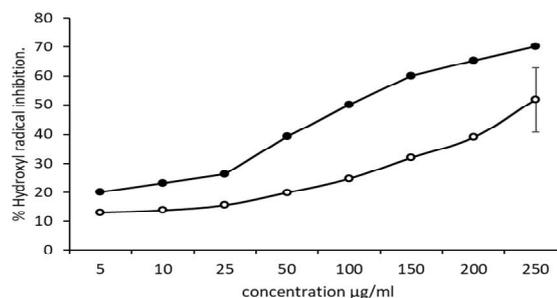


Figure 6: Hydrogen peroxide scavenging activity of UHSEE. **Key:** -○-, UHSEE; -●-, Ascorbic acid

Table 1: Effect of UHSEE on HRBC- membrane stabilization

Treatment	Concentration (µg/mL)	Inhibition (%)
Extract	5	11.76 \pm 0.10
	10	24.89 \pm 0.16
	25	29.67 \pm 0.21
	50	45.78 \pm 0.14
	100	54.37 \pm 0.14
	150	61.49 \pm 0.14
	200	68.92 \pm 0.14
	250	71.32 \pm 0.14
Diclofenac	250	64.49 \pm 0.13

Data are expressed as mean \pm SD (n = 3)

Table 2: Effect of UHSEE on heat induced haemolysis

Treatment	Concentration (µg/mL)	Inhibition (%)
Extract	5	23.45 \pm 0.65
	10	25.55 \pm 0.25
	25	35.08 \pm 0.20
	50	38.55 \pm 0.30
	100	43.74 \pm 0.16
	150	49.78 \pm 0.85
	200	61.90 \pm 0.90
	250	72.67 \pm 1.64
Diclofenac	250	65.86 \pm 0.30

Data are expressed as mean \pm SD (n = 3)

DISCUSSION

The ethanol extract of *Uromastix hardwickii* skin (UHSEE) inhibited the activities of α -glucosidase and α -amylase and increased glucose uptake by yeast cells in a concentration-dependent manner. UHSEE also scavenged reactive oxygen, hydroxyl and NO radicals as well as inhibited the haemolysis of HRBC induced by heat and hypotonic saline.

The inhibition of α -glucosidase and α -amylase activities by UHSEE suggests that it has the capacity to delay carbohydrate digestion and glucose absorption, which will significantly reduce the postprandial increase in blood glucose. The regulation of postprandial hyperglycemia in diabetic patients is severely impaired due to compromised insulin secretion or

insulin resistance [23]. Agents such as acarbose and miglitol which inhibit α -glucosidase and α -amylase activities are useful in the management of type 2 diabetes mellitus [24]. The results of this study are in agreement with the report of Gopal *et al* [25] on the antidiabetic activities of *Lactuca sativa*.

The UHSEE enhanced glucose uptake by yeast cells indicates that it might promote glucose uptake by peripheral cells [18]. Increase in the uptake of glucose by skeletal and hepatic cells is the mechanism of action of metformin. This indicates that UHSEE and metformin may possess similar mechanisms of action.

The inhibitory activity of UHSEE on reactive oxygen species and NO radicals imply that it has antioxidant property that can alleviate oxidative stress implicated in the development and progression of diabetes mellitus and its complications [4]. The finding of this study is in agreement with the report of Da Cunha *et al* [26] on the antioxidant and antidiabetic potentials of *Apis mellifera*.

Membrane stabilization test was used to evaluate the anti-inflammatory potential of UHSEE. It significantly inhibited the haemolysis of HRBC in concentration-dependent manner, in both hypotonic saline and under heat stress. This suggests that UHSEE possesses anti-inflammatory properties. UHSEE may have stabilized the membrane of HRBC by inhibiting the rupture and release of lysosomal enzymes [22], which normally accompanies the inflammatory process. The anti-inflammatory activity of UHSEE observed in the present study corroborates the earlier work of Ferreira *et al* [27] on zoo therapeutics used in Brazilian traditional medicine. The anti-inflammatory property of UHSEE could be useful in the treatment of diabetes, which is essentially an inflammatory process.

CONCLUSION

The ethanol extract of *Uromastix hardwickii* skin (UHSEE) possesses good antidiabetic, antioxidant and anti-inflammatory properties *in vitro*. The findings lend some support for the use of UHSEE for treating diabetes in traditional medicine in Pakistan.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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